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Characteristics of *Se'i* (Rotenesse Smoked Meat) Treated with Coconut Shell Liquid Smoked and *Citrus aurantifolia* Extract

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ABSTRACT

The objective of this study was to investigate the effect of *Citrus aurantifolia* extract (CAE), coconut shell liquid smoke (CSLS) and the combination of CAE and CSLS (CACS) on *se'i* characteristics. A completely randomized design was assigned in this experiment. Treatments used were: *se'i* treated with 5% (v/v) CAE, CSLS 5% (v/v), (CAE : CSL 1:1))/ (CACS) and untreated *se'i* as a control (C). Parameters measured were: aroma, color, taste, pH, residual nitrite, total bacterial count, Coliform, *Staphylococcus aureus, Escherichia coli,* and *Salmonella.* The data of aroma, color, and taste were analyzed by using Kruskal-Wallis test followed by Mann-Whitney test. The pH, residual nitrite, and bacterial data were analyzed with analysis of variance (ANOVA) followed by least significant differences test. Results showed that CAE caused the highest score at both aroma and taste (P<0.05). CSLS caused the lowest residual nitrite (27 ppm) (P<0.05). Application of CAE and CACS could reduce total bacteria (P<0.05) at least 1 log. Color, pH, and Coliform number were not significantly different. *S. aureus, E. coli,* and *Salmonella* were negative in all *se'i* samples. CAE gives the best organoleptics and bacteriological characteristics while CSLS is more effective in reducing nitrite.

Key words:** Citrus aurantifolia **extract, coconut shell liquid smoke,** se'i, **smoked beef

ABSTRAK

Tujuan penelitian ialah untuk menguji pengaruh penggunaan ekstrak jeruk nipis (*Citrus aurantifolia*) (EJN), asap cair tempurung kelapa (ACTK), dan gabungan pemberian EJN dan ACTK (EJTK) terhadap karakteristik daging *se'i* (daging asap Rote). Penelitian ini menggunakan rancangan acak lengkap (RAL) dengan 4 perlakuan, yaitu *se'i* yang tidak mendapat perlakuan sebagai kontrol, *se'i* yang diberi EJN 5% (v/v), *se'i* yang diberi ACTK 5% (v/v), dan *se'i* yang diberi EJTK (EJN:ACTK 1:1). Setelah daging *se'i* dicampur dengan garam dan sendawa (KNO_3), *se'i* diberi EJN, ACTK, dan EJTK sesuai perlakuan yang disebut di atas. Campuran tersebut diperam sekitar 12 jam kemudian diasapi. Peubah yang diukur ialah aroma, warna, rasa, pH, residu nitrit, total bakteri, Coliform, *Staphylococcus aureus, Escherichia coli,* dan *Salmonella.* Data aroma, warna, dan rasa dianalisis menggunakan analisis nonparametrik Kruskal-Wallis dilanjutkan dengan uji Mann-Whitney. Data pH, residu nitrit, dan bakteria dianalisis menggunakan analisis varians (ANOVA) dan uji beda nyata terkecil (BNT). Hasil penelitian ini menunjukkan bahwa skor aroma dan rasa tertinggi adalah pada *se'i* yang diberi EJN (P<0,05). Residu nitrit terendah (27 ppm) pada *se'i* yang diberi ACTK. Pemberian EJN dan EJTK menyebabkan penurunan total bakteri (P<0,05) sedikitnya 1 log. Skor warna, nilai pH, dan jumlah bakteri Coliform pada semua sampel *se'i* tidak berbeda dan berada pada standar normal kualitas daging *se'i*. *S. aureus, E. coli,* dan *Salmonella* terdeteksi negatif pada semua sampel *se'i*. Penelitian ini menyimpulkan bahwa pemberian EJN meningkatkan skor organoleptik dan menurunkan total bakteri. Perlakuan ACTK lebih efektif menurunkan residu nitrit pada daging *se'i*.

***Kata kunci:** ekstrak jeruk nipis, asap cair tempurung kelapa,* se'i, *daging asap*

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INTRODUCTION

Se'i is a Rotenese traditional processed ready-to-eat smoked meat product. Rote is a small island located in the west part of Indonesia that is in border with Australia. *Se'i* is made from boneless beef, pork, or deer meat. *Se'i* processing is begun with the meat is sliced into pieces with ± 3 cm of thickness from one side to other side of the meat so it has the rope- shape. The meat is spiced with salt and saltpeter (KNO_3) and then cured for ± 12 h. The rope-shape meat was then arranged above the fire place for smoking until well done.

As a traditional product, *se'i* has a good prospect for culinary marketing in the future. However, recently quality of *se'i* as smoked meat gets seriously attention because of the growing concern among consumers about such carcinogenic components which always contain in smoked meat. When the meat is placed directly upon the frame to be smoked, all components in smoke vapor will adhere directly to the meat surface. Meanwhile in the smoke contains both desirable and undesirable components. Undesirable components such as tars and polycyclic aromatic hydrocarbons (PAH) are recognized as being carcinogens. Moreover using wood as a fuel in smoking processing is quite difficult since it takes time and result of organoleptic characteristic is vary. First grade of liquid smoke, on the other hand, have some advantages such as easy to be applied, uniformity of the product, and the smoke products more clean. In coconut shell liquid smoke, components which are recognized as being carcinogens and always found in gas smoke, such as tars and polycyclic aromatic hydrocarbons (PAH), are not found (Budijanto *et al*., 2008).

The traditional (vaporous) smoking of *se'i* is usually done by *Schleichera oleosa* wood smoke and above the meat surface is covered with *S. oleosa* raw leafs. Nowadays *S. oleosa* tree has been prohibited to be cut since the habitat of the tree almost extinct. Other wood smokes that could be used to smoke *se'i* is coconut shell. However, it causes bitter in taste since the heat of coconut shell is too strong, thus application of coconut shell liquid smoke is prefer.

Several studies of the effect of liquid smoke were applied in several kinds of foods in the world such as color preservatives in raw tuna and salmon fish (Schubring, 2008), suppressed the growth of *Listeria monocytogenes* (Lm) for up to 130 d in frankfurters (Martin *et al*., 2010), reduced TBA numbers, aroma score and pH values in ground beef (Estrada-Munoz *et al*., 2008), increased aroma score in beef (Arizona *et al*., 2011), prolong shelf life of fish ball (Zuraida *et al*., 2011), stabilized oxidation of catfish sausage (Ernawati *et al*., 2012), and decreased cut off strength of broiler meat (Yosi & Sandi, 2014). Liquid smoke was also effective against various types of spoilage and pathogenic microorganisms (Milly *et al*., 2005).

Application of liquid smoke in meat can be used as a single treatment or is mixed with citric or acetic acids (Pearson & Gillett, 1996). The combination of liquid smoke and citric acid give double benefits of the liquid smoke and the acid when be applied to *se'i*. Since price of liquid smoke is expensive while source of acids could be obtained from fresh citrus fruits that means is cheaper.

Using of *Citrus aurantifolia* could reduce lactic acid bacteria and *Listeria* in beef processing (Fernandez-Lopez *et al*., 2005), increased meltability and elasticity of Mozzarella cheese (Purwadi, 2007), reduced residual nitrate level in cured meat (Ermawati, 2008) and increased level of water, carbohydrate and protein in cooked rice (Haq *et al*., 2010). Thus, addition of *C. aurantifolia* extract probably could change sensory attributes of *se'i*.

Traditionally, kinds of nitrate salt added in *se'i* (smoke beef) processing is KNO_3. Nitrate could result in formation of carcinogenic n-nitrosamines in cured meat, thus *C. aurantifolia* ability to reduce residual nitrite level could avoid the formation of nitrosamines and nitrosamides (Viuda-Martos *et al*., 2009). Organoleptics aspect, bacterial number, and residual nitrite in *se'i* given liquid smoke or *C. aurantifolia* extract has not been reported yet.

The aim of this study was to compare the effect of coconut shell liquid smoke, *C. aurantifolia* extract, and their combination on organoleptic characteristics, pH value, residual nitrite, and antibacterial activity of *se'i* (*Rotenese smoked beef*).

MATERIALS AND METHODS

A total of 22 kgs of beef was obtained from butt and rump of Bali cattle bought in meat shop in Kupang. Coconut shell liquid smoke (CSLS) was obtained from Department of Technology Agriculture, Gadjah Mada University. The fruits of *C. aurantifolia* were washed with distilled water, sliced around from the top to the bottom and then squeezed to obtain the extract. To obtain 5% (v/v) concentration of the extract or the liquid smoke, 5 mL of the extract or the liquid smoke were poured into 350 mL volumetric glass and then added distilled water to bring the volume to about 100 mL. The mixture was heated at 45 °C in a stirring hot plate for 30 min, filtered with Whatman (No. 41) filter paper. Then stored at 4 °C for 2 d (until used).

Treatments and *Se'i* Processing

Completely randomized design (CRD) with 4 treatments was assigned in this experiment. Beef were trimmed off excessive of connective tissue and fat, sliced in rope-shape (*lalolak*). For each kg of beef, 500 mg of saltpeter (KNO_3) and 2% of salt were added and mixed well manually. Total of 22 kgs of the beef were divided into four groups as treatment given. The first group was untreated *se'i* as control (C). The second group was subjected to 5% (v/v) of coconut shell liquid smoke (CSLS), the third group was treated with 5% (v/v) of *C. aurantifolia* extract (CAE), and the fourth group was added 5% (v/v) of coconut shell liquid smoke + 5% (v/v) of juice of *C. aurantifolia* (CACS). Each group of beef was mixed well and marinated for ± 12 h.

The marinated beef from the first group (C) and the third group (CAE), without liquid smoke, were smoked traditionally. While the second (CSLS) and the fourth group (CACS), treated with liquid smoke, were

smoked in oven by using Hock stove with 22 wicks. All meat surface was covered with *S. oleosa* leaf while smoking, following the way of traditional *se'i* processing. Then *se'i* was placed into polyethylene plastic bags, vacuumed with house-hold vacuum sealer Dhromex, and stored at cold temperature (4 °C) for 4 d. At the fifth day, the packages were analysed.

Se'i analysis included organoleptic aspects: taste, aroma, and color. Other parameters measured were pH, residual nitrite, and the bacterial numbers: total bacteria, *coliform, Escherichia coli, Staphylococcus aureus*, and *Salmonella*.

Sensory Analysis

A nine members of trained panelists from Animal Science Faculty students were selected for sensory analysis (color and aroma). At training time, these panelists were given 5 pieces of high-quality commercial *se'i* taken from local market to familiarize the panelists about color and aroma of *se'i* products.

At evaluation time, each panelist was given four uncoded *se'i* samples and asked to evaluate the taste, aroma, and color. Panelists evaluated sensory attributes twice for each replicate. Evaluation was initiated after the panelists agreed on the specifications. The samples were served to each panelist separately. Panelists were asked to clean their palates between samples with water. The color and aroma were examined by using scoring test. The color score was: 5= very bright red color; 4= bright red color (specific *se'i* color), 3= dark red, 2= moderately dark red, 1= light red. To examine the aroma 30 g of samples were sliced, put into small glass jars, and allowed to stand for several hours (Bensink *et al*., 1973). On opening the jars, the panelists immediately examined the aroma by assigning score 1= no aroma to 5= very strong aroma.

The panelists were asked to examine the organoleptics (taste) on the score sheet for hedonic-scale rating test. The taste score was 5= like very much, 4= like moderately, 3= like slightly, 2= dislike moderately a 1= dislike very much. For each treatment, each penelist had three pieces of *se'i* to be evaluated. An average of the three measurements to calculate mean score for each of the color, aroma, and taste of *se'i* sample.

Determination of pH

Determination of pH value was measured by using a Hanna digital pH-meter at ambient temperature. A 10 g of *se'i* was minced and then homogenized with 10 mL distilled water and filtered (with Whatman paper no 4). The pH meter was standardized between pH 4.0 and 7.0. The filtrate pH obtained was recorded by inserting the probe of the pH meter into the filtrate for 30 s then the value was read. For each treatment, measurements were made in triplicate.

Residual Nitrite Analysis

Residual nitrate level of *se'i* was determined as mg $NaNO_2$ kg^{-1} meat by a spectrophotometer method at 540 nm as described in AOAC (1995). For each treatment, measurements were made in duplicate.

Around 5 g of *se'I* was weighed, minced and transferred into a 250-mL beaker. Forty milliliter of water was added and heated to 80 °C for 15 min then the mixture was transferred into a 250-mL volumetric flask. Hot water was added to bring the volume to about 200 mL and the flask was transferred to steam bath for 2 h shaking occasionally. The solution was cooled to room temperature. The volume was completed to 250 mL with water, filtered, and centrifuged to clear the solution. Two and a half milliliters of sulphanilamide solution was added to aliquot containing 5-50 ug $NaNO_2$ in 50 mL vol flask and the the solution was mixed. Two and a half milliliters of NED reagent was added and 5 min later, the solution was mixed and the colour was allowed to develop for 15 min. A 5-mL portion of solution was transferred to photometer cell and absorbance was determined at 540 nm against blank of 45 mL water and 2.5 mL of sulphanilamide reagent, and 2.5 mL of NED reagent. The concentration of nitrite was determined by comparison with standard curve with straight line up to 1 ppm $NaNO_2$ in final solution. To make standard curve; 10, 20, 30, and 40 mL of nitrite working solution were added to 50 mL vol flasks. Two and a half milliliters of sulphanilamide reagent was added and after 5 min, 2.5 mL of NED reagent was added.

Bacterial Analysis

Bacterial analysis were carried out following the procedure of Harrigan and McCance (1976). In each case, 10 g of each sample for microbiological evaluation were aseptically transferred into 90 mL of 0.1% sterile peptone water, shaken thoroughly and appropriate dilutions (up to 10^5). Total viable counts (aerobic mesophiles) were made on Plate Count Agar (PCA, Oxoid, U.K.). Coliforms were isolated by using MacConkey broth and Eosin Methylene Blue agar. A 10-1 dilution of each sample was enriched in tetrathionate broth (Difco), incubated at 37 °C for 6 h before inoculation on *Salmonella-Shigella* agar (Oxoid) for isolation of *Salmonellae*. Typical colonies of Coliform were identified as round, red to pink, 0.5-2 mm in diameter, surrounded with a red to pink halo. *Staphylococcus* strains were isolated on Mannitol salt agar and Baird-Parker medium for *S. aureus*, incubated at 37 °C for 24 to 48 h (Elliot *et al*., 1978). All typical colonies on Baird-Parker agar were counted. The coagulase and catalase tests were used to differentiate *S. aureus* from other Staphylococci. For each treatment, measurements were made in triplicate.

Statistical Analysis

The data of color and aroma were analyzed by using non parametric, Kreskas-Wallis test. Mann-Whitney test was used to test for difference between means (significance $P<0.05$; highly significant was $P<0.01$). The pH, residual nitrite, and bacterial data were analysed with analysis of variance (ANOVA). Least significant differences test was used to determine the differences

among means (significantly different was P<0.05; highly significant was P<0.01) (SPSS, 18).

RESULTS AND DISCUSSION

Sensory Characteristics

The taste and aroma of *se'i* that had treatments were significantly different from control *se'i* (P<0.05), however color was not significantly different from *se'i* control (P>0.05). Table 1 showed that CAE caused the highest score at both taste and aroma of *se'i* samples while the control had the lowest score.

The juice of *C. aurantifolia* was squeezed from the fruit contained the most citric acid (46 g/L) (Penniston *et al*., 2008). Citirc acid had sour in taste. Giving CAE caused *se'i* has little sour in taste and panelists preferred to the taste compared to other *se'i* products in this experiment.

Budijanto *et al*. (2008) reported that the two main compounds contain in CSLS were guaiacol and its derivatives (36.58%) and phenol and its derivatives (24.11%). According to Varlet *et al.* (2007a) the phenol and guaiacol were mainly associated with aroma and flavour of smoked products. It was indicated that in this experiment the two compounds had a little influence in taste and aroma of *se'i* compared to citric acid in the CAE, eventhough CSLS caused higher score compared to control.

Color intensity in smoking product mainly depend on carbonyl-containing compounds that reacts with amino acids to form golden-brown color of smoked products, Maillard – type reaction (Varlet *et al*., 2007b). In CSLS contained carbonyls and acids (2.98%) (Budijanto *et al*., 2008). While in CAE commonly contain ascorbic acid, malic acid, and citric acid. Ascorbic acid in CAE can accelerate the conversion of nitrite to nitric oxide (NO) thus could promote cured color formation (Gøtterup *et al*., 2008).

In cured meat, when nitrate was given, it was converted to nitrite by nitrate – reducing bacteria and then nitrite was reduced to nitric oxide (NO) that reacted with myoglobin to form nitric-oxymyoglobin, red in color but unstable. When the meat was smoked, nitric-oxymyoglobin was converted to Nitrosylhemochromagen that was responsible for stable cured-pink color (Sebranek & Bacus, 2007) that was a specific color of *se'i*. Table 1 revealed that there was no significant difference between treated samples, and all the color score was normal color of *se'i*. Although CSLS could reduce more nitrite in *se'i* (Table 2), the same effect in *se'i* indicated that carbonyls in CSLS and ascorbic acid in CAE had the same effect on *se'i* color.

pH Value

Acids contained in substances can influence the pH value of *se'i*. CAE contains citric acid, malic acid, and ascorbic acid (Güçlü *et al*., 2005) whereas CSLS contains formic, acetic, propionic, and butyric acids (Pearson & Gillet, 1996). Although that kind of organic acids contained in CAE and CSLS were different, addition of CAE, CSLS, and their combination could not alter the pH value of *se'i* (Table 2). However, the pH values of *se'i* samples were below the critical limit value of 7.0, which indicated the ability of CAE, CSLS, and CACS to inhibit or reduce the development of bacteria in *se'i*.

Residual Nitrite

In *se'i* processing, nitrate/saltpeter added for the purpose of curing will be found in the finished product as residual nitrite. In this experiment, the residual nitrite levels (ppm) of *se'i* samples were shown in Table 2. Addition of CSLS, CAE, and CACS could reduce residual nitrate level in *se'i* compared to control (P<0.05) and the lowest residual found in *se'i* sample treated with CSLS.

The means residual nitrite permitted in processing meat is 30 mg/kg (Indonesian Food and Drugs Board, 2013). In this experiment the only *se'i* sample fixed to the rule was *se'i* treated with CSLS. Level of nitrate used in this experiment was 500 mg/kg of beef. When nitrate was added to meat, this compound would be converted to nitrite and nitrite reacts with oxygen to form nitric oxide (NO). It is about 5%-15% NO bound with myoglobin, 5%-15% bound with sulfhydryl group, 1%-5% bound with lipid, and 20%-30% bound with protein. Thus the amount of nitrate left after processing only less than 50% (Cassens *et al*., 1976). In this experiment the amount of nitrate left range from 5.4%-85.4%. Residual nitrite in *se'i* could be decreased by reducing the initial nitrate level. In *se'i* processing, potassium nitrate/saltpeter (KNO_3) is added as preservative and for color development. Nitrate could inhibit outgrowth and neurotoxin formation by *C. botulinum* (Yetim *et al*., 2006) and could help to form typical red or pink color of cured meat (Gøtterup *et al*., 2008). Various factors influence the

Tabel 1. Score of taste, aroma, and color of se'i treated with coconut shell liquid smoke (CSLS), *Citrus aurantifolia* extract (CAE), and combination of coconut shell liquid smoke and *C. aurantifolia* extract (CACS)

Treatment	Control	CSLS	CAE	CACS
Taste	3.90±0.01[a]	4.30±0.02[b]	4.70±0.01[c]	4.10±0.02[b]
Aroma	3.60±0.01[a]	3.90±0.02[b]	4.70±0.01[c]	4.20±0.01[b]
Color	3.28±0.02[a]	4.32±0.01[a]	4.20±0.02[a]	4.34±0.01[a]

Note: Means in the same row with different superscripts differ significantly (P<0.05).

Tabel 2. pH value and residual nitrite (ppm) of se'i treated with coconut shell liquid smoke (CSLS), *Citrus aurantifolia* extract (CAE), and combination of coconut shell liquid smoke and *C. aurantifolia* extract (CACS)

Treatment	Control	CSLS	CAE	CACS
pH	5.63±0.01[a]	5.59±0.03[a]	5.50±0.03[a]	5.63±0.01[a]
Residual nitrite (ppm)	427.00±0.10[a]	27.00±0.05[b]	384.00±0.01[c]	236.00±0.12[d]

Note: Means in the same row with different superscripts differ significantly (P<0.05).

Table 3. The number of total bacteria, *Coliform, Staphylococcus aureus, E. coli,* and *Salmonella* (log cfu/g ± SD) of *se'i* treated with coconut shell liquid smoke (CSLS), *Citrus aurantifolia* extract (CAE), and combination of coconut shell liquid smoke and *C. aurantifolia* extract (CACS)

Treatment	Total bacteria (cfu)	*Coliform* (mpn/g)	*Staphylococcus aureus*	*E. coli*	*Salmonella*
Control	4.87±0.22[a]	0.32±0.12	Negative	Negative	Negative
CSLS	4.66±0.11[a]	0.01±0.01	Negative	Negative	Negative
CAE	3.48±0.12[b]	0.56±0.01	Negative	Negative	Negative
CACS	3.87±0.01[b]	0.56±0.01	Negative	Negative	Negative

Note: Means in the same column with different superscripts differ significantly (P<0.05).

rate of nitrite reduction such as pH, the presence of reductants, initial nitrate level (Pérez-Alvarez *et al*, 1993 in Viuda-Martos *et al*., 2009) heating process, and storage time (Sebranek & Bacus, 2007). In this study, pH of all *se'i* samples, initial nitrate level and storage time were same thus the effect of the three factors on reducing nitrite were neglected.

The lowest residual nitrite was reached when *se'i* was treated with CSLS and when CSLS combine with CAE (CACS) the residual nitrite was lower than *se'i* treated with CAE only. It is indicated that the role of reductants such as active biocompounds (polyphenols) (Moawad *et al*., 2012) presented in CSLS (Budijanto *et al*., 2008) is more effective in reducing nitrite in *se'i* compared to ascorbic acid present in CAE.

It is important to keep residual nitrite at low level to prohibit nitrosamine formation in *se'i* product, since nitrosamine seems to be associated with gastric cancer (Jakszyn & Gonzales, 2006; Larsson *et al*., 2006). Nitrosamines are formed when nitrite reacts with secondary amines in food.

Bacterial Characteristic

Bacterial numbers (total bacteria, *coliform*, *S. aureus*, *E. coli*, and *Salmonella)* of *se'i* samples were shown in Table 3. Maximum limitation of bacterial contamination permitted in smoked meat was $1x10^5$ cfu (5 log cfu/g) and 10 mpn for *Coliform* (SNI.7388:2009). Comparing to the data in Table 3 it showed that there was no *se'i* sample in all treatment and control having number of bacteria higher than $1x10^5$cfu. It is indicated that all *se'i* samples exhibit good quality. *S. aureus*, *E. coli,* and *Salmonella* were not detected in *se'i* samples.

Application of *C. aurantifolia* (CAE), coconut shell liquid smoke + *C. aurantifolia* (CACS) could reduce the number of total bacteria (P<0.05) at least 1 log. It seemed that the effectiveness of coconut shell liquid smoke in reducing the total bacteria number increased when it mixed with *C. aurantifolia* extract (CAE), whereas *Coliform* bacteria was same in all *se'i* samples.

It was reported that phenolic, carbonyls, and organic acids compounds contained in liquid smoke had antibacterial activity (Pearson & Gillet, 1996). However, the major contribution came from phenolic group such as phenol, 2-methoxylphenol (guaiacol), 3,4 dimethoxylphenols, and 2-methoxy-4-methyl phenol (Soldera *et al*., 2008). Meanwhile flavonoids and vitamin C are prominent in *C. aurantifolia* and play a major contribution in antibacterial activity (Martin *et al*., 2002). Based on data of Table 3, it was obvious that *C. aurantifolia* had a stronger antibacterial activity compared to liquid smoke. Flavonoids and vitamin C might enhance the effectiveness of phenols as antibacterial activity when CSLS and CAE applied together (CACS).

CONCLUSION

The taste, aroma, and bacterial characteristics of *se'i* treated with *C. aurantifolia* extract (CAE) would appear to be better when treated with coconut shell liquid smoked (CSLS). Antibacterial effect of CSLS increased when combined with CAE (CACS). *S. aureus*, *E. coli* and *Salmonella* were not detected in all *se'i* samples. The color and pH of *se'i* were not affected by treatments. CSLS was the best treatment in reducing nitrite in *se'i*.

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